STIC Search Report
Biotech-Chem Library

STIC Database Tracking Number: 169018

TO:	From:
Ilia Ouspenski **Location: REM-3D74&3C70** **Art Unit: 1644** **Thursday, October 20, 2005** **Case Serial Number: 10/625105**	**Deirdre Arnold** **Location: Biotech-Chem Library** **REM 1A64** **Phone: 571-272-2532** **Deirdre.Arnold@uspto.gov**

Search Notes

RUSH

Please feel free to contact me if you have any questions or would like to amend the search.

Thank you for using STIC services.

Regards,
Deirdre Arnold

STIC-Biotech/ChemLib 169018

From: Chan, Christina
Sent: Wednesday, October 19, 2005 2:51 PM
To: Ouspenski, Ilia; STIC-Biotech/ChemLib
Subject: RE: RUSH sequence search request for 10/625,105

IRFE

Please rush. Thanks Chris

Chris Chan
TC 1600 New Hire Training Coordinator and SPE 1644
(571)-272-0841
Remsen, 3E89

-----Original Message-----
From: Ouspenski, Ilia
Sent: Wednesday, October 19, 2005 2:29 PM
To: Chan, Christina
Subject: RUSH sequence search request for 10/625,105

Christina, please approve a RUSH search:

STIC:

please provide an INTERFERENCE ONLY search for amino acid sequences SEQ ID NOS: 28, 30, 32, 34, 36, and 38 for 10/625,105.

Thnaks,

ILIA OUSPENSKI, Ph.D.
Examiner
Art Unit 1644
Phone:571-272-2920
REM 3D74
Mailstop 3c70

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Searcher:_____
Searcher Phone:_____
Date Searcher Picked up:_____
Date completed:_____
Searcher Prep Time:_____
Online Time:_____

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Type of Search
NA#_____ AA#:_____
S/L:_____ Oligomer:_____
Encode/Transl:_____
Structure #:_____Text:____
Inventor:_____ Litigation:___

****
Vendors and cost where applicable
STN:_____
DIALOG:_____
QUESTEL/ORBIT:_____
LEXIS/NEXIS:_____
SEQUENCE SYSTEM:_____
WWW/Internet:_____
Other (Specify):_____

Tezuka et. al., Poster, 1994.

OBJECTIVE: Recently, adhesion molecules in immune system cells have been identified and their functions have been clarified, but when one considers the complex nature of the immune system and tissue specificities and so forth, it is presumed that unknown adhesion molecules exist. The JTT.1 antibody, which we obtained in the course of searching for novel adhesion molecules, has a function to aggregate rat thymoma cell line FTL435 cells via an unknown adhesion pathway. Therefore it was believed the antigen molecule recognized by the JTT.1 antibody was a signal transduction molecule that induces an unknown cell adhesion. This time we produced the antibody JTT.2 with adhesion blocking activity and cloned the JTT.1 antigen gene in order to further clarify the properties of this adhesion phenomenon. As a result, we report that it was clarified that the JTT.1 antigen not only functions as a signal transduction molecule, but is also an adhesion molecule.

METHOD: Antibody preparation: Made by immunizing BALB/c mice with rat thymoma cell line FTL435, and fusing lymph node cells with mouse myeloma cells. Immunoprecipitation: Performed by making biotinylated cells soluble and using antibody-binding beads. Precipitates were subjected to SDS-PAGE, then transferred to a film and detected using an ECL system. Genetic cloning: poly(A)$^+$ RNA was prepared from rat ConA blast, cDNA was synthesized with this as a template, and then it was incorporated into an expression vector and a cDNA library was constructed. The library was transiently expressed with COS cells, and screened by panning using the JTT.1 antibody. The cDNA sequences concentrated by panning were determined by the dideoxy method. Flow cytometry analysis: Cells were treated with the antibody, stained with FITC-conjugated anti-mouse Ig, and then analyzed by flow cytometry.

RESULTS AND CONSIDERATIONS: A mouse was immunized with FTL435 cells, monoclonal antibodies were prepared in accordance with an index for blocking aggregation of the FTL435 cells by JTT.1 antibody, and JTT.2 was obtained. The isotype of JTT.2 antibody was IgG1κ. The JTT.2 antigen was strongly expressed in FTL435 cells, thymocytes, and activated lymphocytes. The result of immunoprecipitation indicated that the molecular weight of the JTT.2 antigen molecule was 24 kDa and 28 kDa by reduction. This matched the case of the JTT.1 antigen's molecule. Based on this, it was believed the JTT.2 antibody recognized the JTT.1 antigen. Therefore we performed an experiment to bind rat thymocytes to the JTT.1 antigen immobilized on a plate. Thymocytes in an unstimulated state did not bind to the JTT.1 antigen, but bound specifically to the JTT.1 antigen on a plate when stimulated with the JTT.1 antibody. This adhesion phenomenon was blocked by adding the JTT.2 antibody. Based on these results, it was believed the JTT.1 antigen is an adhesion molecule.

Next, we performed genetic cloning of the JTT.1 antigen. A cDNA library was prepared from rat ConA blasts that express the JTT.1 antigen. The cDNA library was transiently expressed in COS cells, and panning using the JTT.1 antibody was repeated three times, thus cloning a 0.9 kbp gene. COS cells that transiently expressed this gene reacted strongly with the JTT.1 and JTT.2 antibodies. A sequence matching the N-terminal sequence of the purified JTT.1 antigen was present in the amino acid sequence predicted from the nucleotide sequence. Based on these results, it was believed that the gene we cloned was the JTT.1 antigen gene. In the predicted amino acid sequence there

were sugar-chain binding sites at two positions in the extracellular domain, and, in the intracellular domain, there was a PKC phosphorylation site at one position and CK2 phosphorylation sites at two positions. Furthermore, we performed homology searching for the entire length of the amino acid sequence, but no homologous molecule among previously known molecules was found. Therefore it is believed the JTT.1 antigen is a novel adhesion molecule.

SUMMARY

1. A JTT.2 antibody that blocks unknown cell aggregation induced by the JTT.1 antibody was prepared.
2. It was believed the JTT.2 antibody recognizes the JTT.1 antigen.
3. Rat thymocytes stimulated by the JTT.1 antibody bound to the JTT.1 antigen. This binding was specifically blocked by the JTT.2 antibody.
4. The JTT.1 antigen gene was obtained by expression cloning. Homology searching resulted in finding no highly similar molecule.

Based on these results, it is believed the JTT.1 antigen is a novel adhesion molecule.

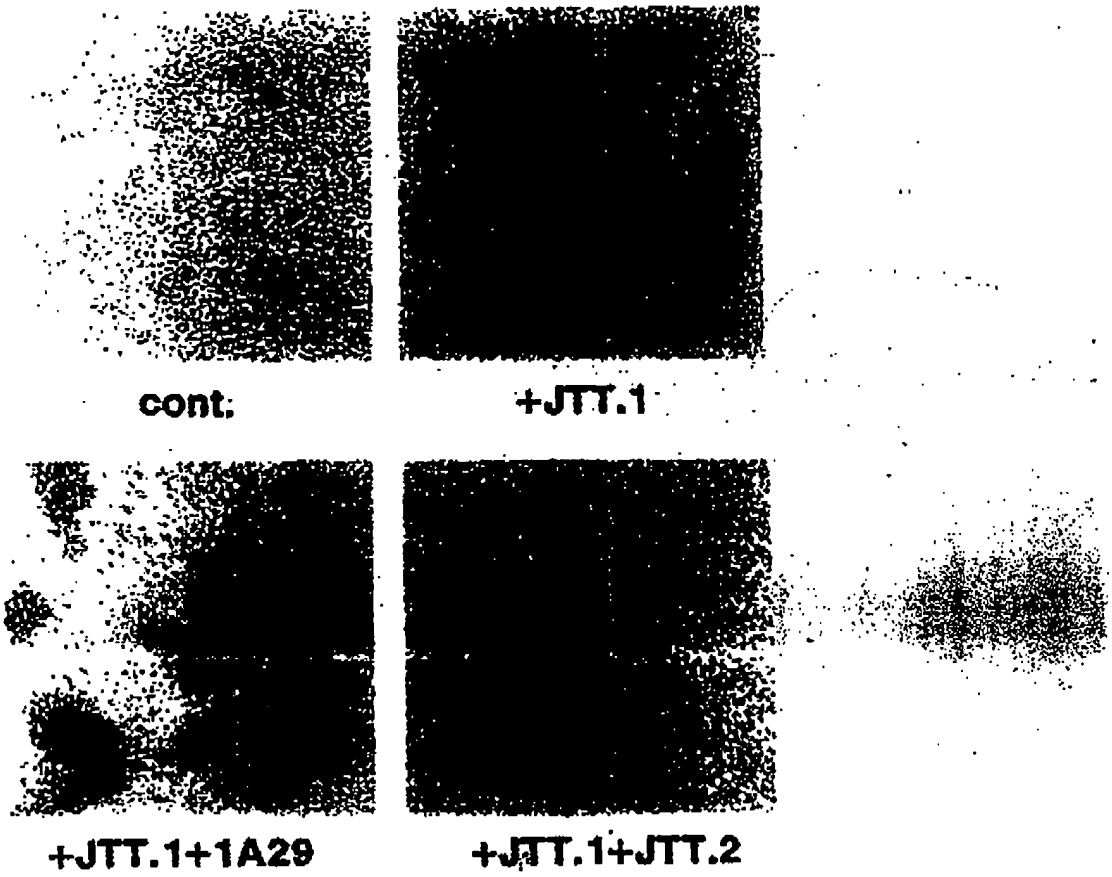


FIG. 1
JTT.2 antibody's FTL435 cellular aggregation blocking activity.
FTL435 cells were cultured at 37 degrees C for one hour in the presence or absence of the antibody.

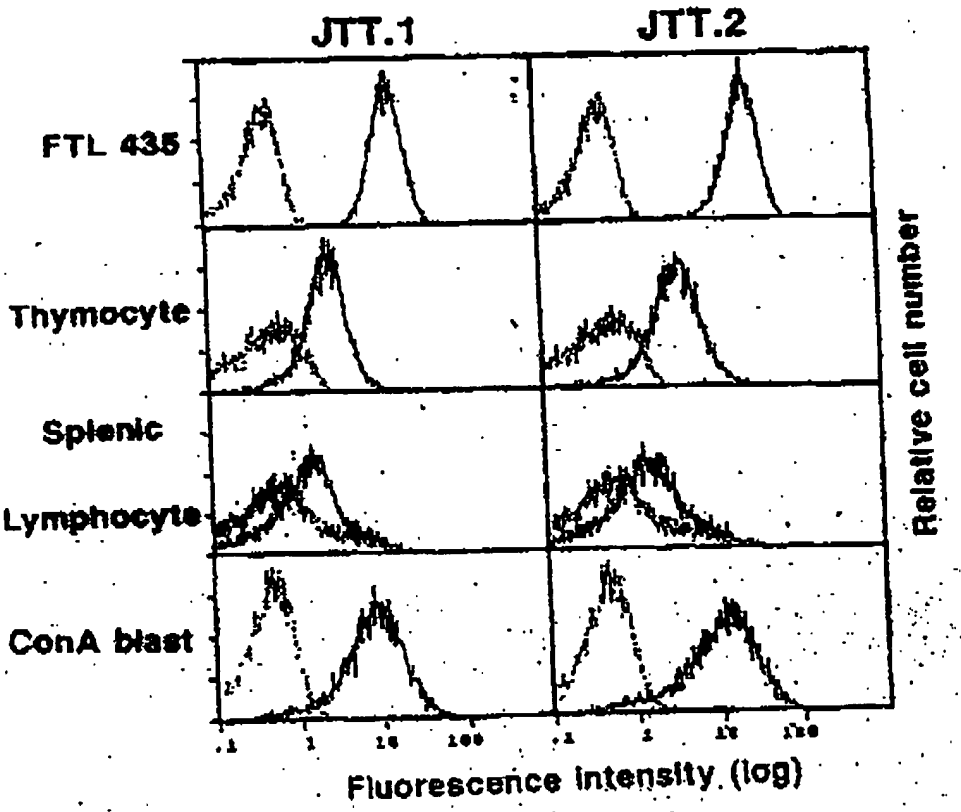


FIG. 2
Comparison of expression patterns of JTT.1 antigen and JTT.2 antigen in various cells.
FTL435, thymocytes, splenic lymphocytes, and ConA blasts were stained with antibodies and analyzed with flow cytometry.

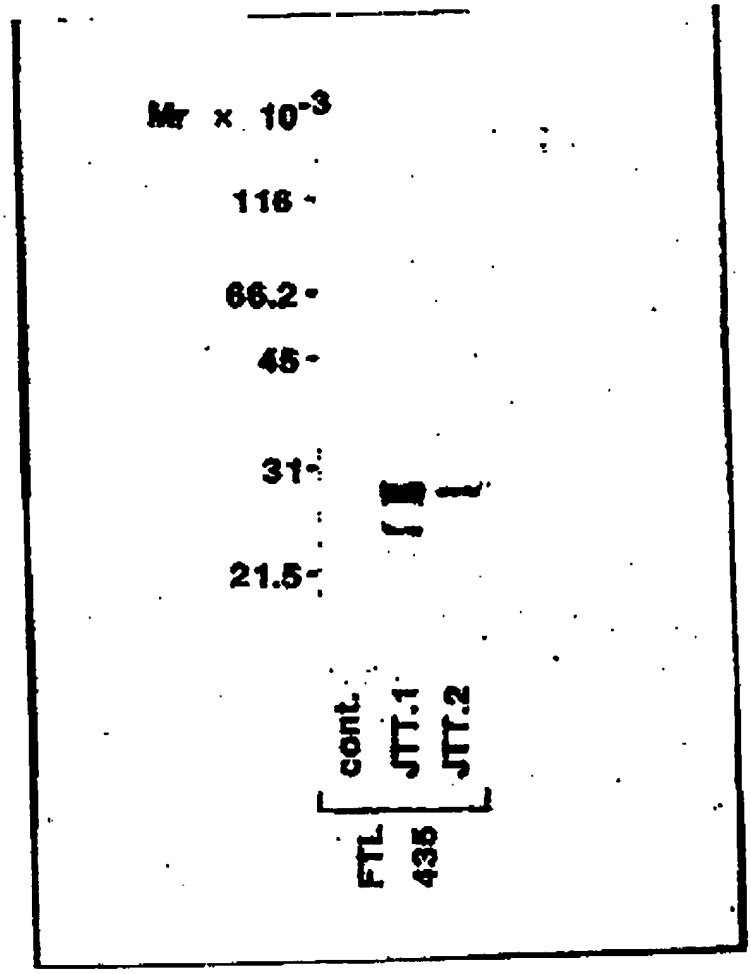


FIG. 3
Comparison of JTT.1 antigen and JTT.2 antigen according to immunoprecipitation.
Each antigen was immunoprecipitated using the soluble material of biotinylated FTL435 cells, subjected to SDS-PAGE, transferred to a film, and detected with an ECL system.

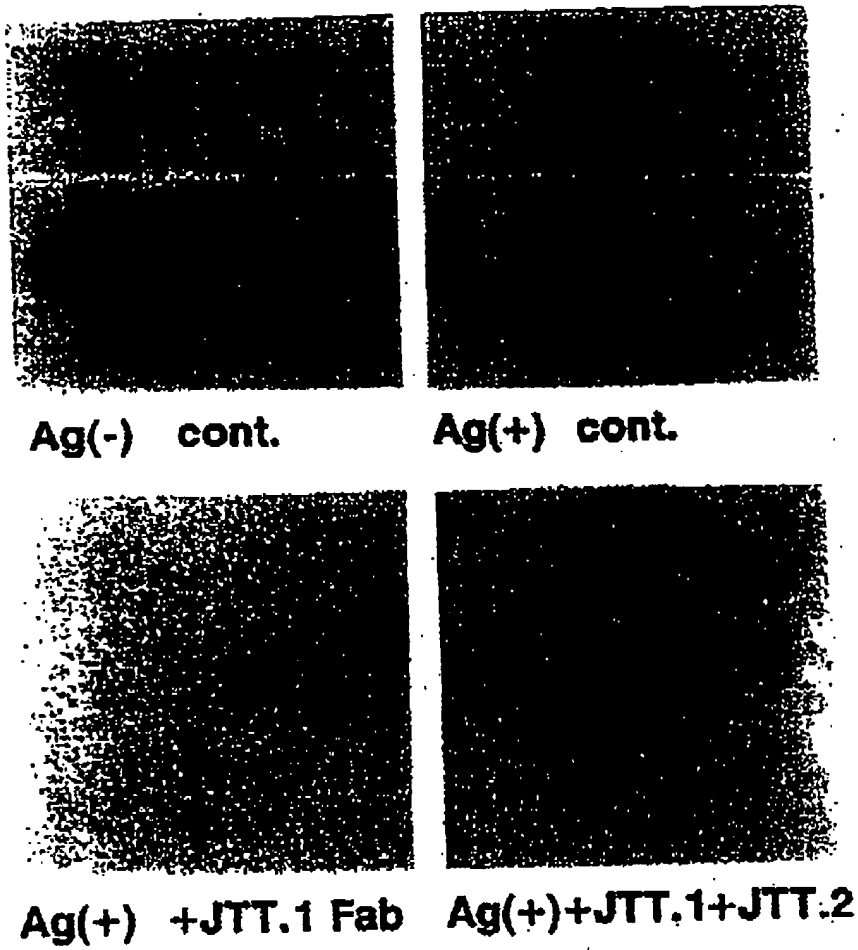


FIG. 4
The JTT.2 antigen blocks binding of thymocytes to the JTT.1 antigen.
Purified JTT.1 antigen was coated on a plate;
rat thymocytes were cultured at 37 degrees C for one hour in the presence and absence of the antibody.

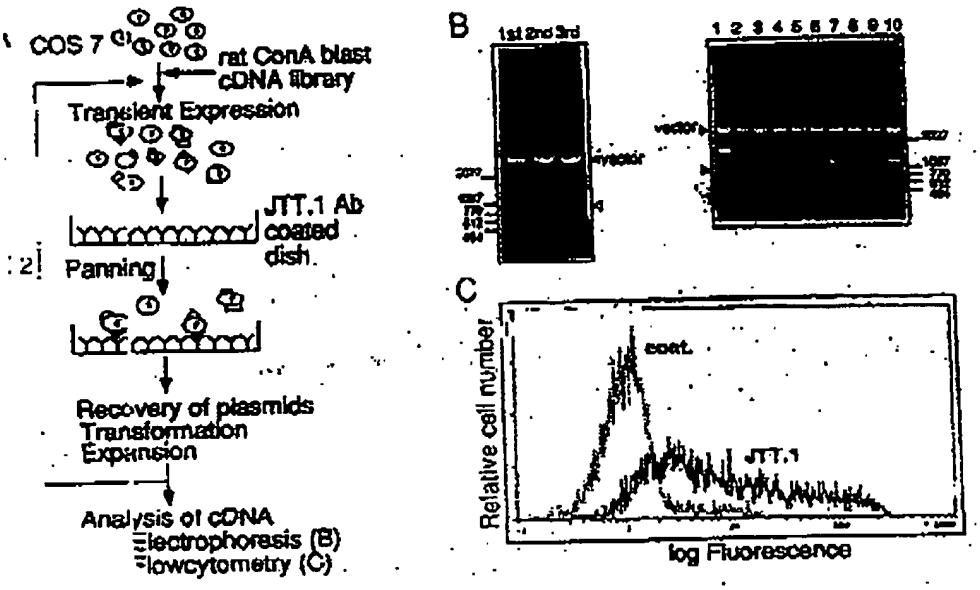


FIG. 5
JTT.1 antigen expression cloning.

(A) Summary of cDNA cloning using panning method.
(B) Analysis of plasmids recovered using COS cells: Restriction enzyme digestion of recovered plasmids, then 1% agarose gel electrophoresis, and analyze insert DNA.
(C) Reactivity of JTT.1 antigen cDNA product with JTT.1 antibody: Use JTT.1 antibody to stain COS cells that transiently expressed JTT.1 antigen cDNA obtained by panning, and analyze with flow cytometry.

【目的】最近、免疫系細胞における接着分子の同定や機能解明が行われているが、免疫系の多様性や組織特異性などを考えると未知の接着分子の存在が予想される。われわれが新たな接着分子を検索する過程で得たJTT.1 抗体は、ラット胸腺腫細胞株 FTL435 細胞を未知の接着経路を介して凝集させる作用を有する。このことから、JTT.1抗体が認識している抗原分子は、未知の細胞接着を誘導するシグナル伝達分子と考えられた。今回、この接着分子の性状をさらに明らかにするために、接着阻害活性を有する抗体JTT.2を作成するとともに、JTT.1抗原遺伝子をクローニングした。その結果、JTT.1 抗原がシグナル伝達分子としての機能を有するだけでなく、接着分子であることが明らかとなったので報告する。

【方法】抗体の作成：ラット胸腺[illegible]FTL435をBALB/cマウスに免疫し、リンパ節細胞をマウスミエローマ細胞と[illegible]合することにより作成した。免疫沈降：ビオチン化した細胞を可溶化し、[illegible]ビーズにより行った。沈降物は、SDS-PAGE後膜に転写し、ECLシステム[illegible]検出した。遺伝子クローニング：ラットCon A blastよりpoly(A)$^+$RNAを[illegible]、これを鋳型としてcDNAを合成後、発現ベクターに組み込みcDNAライブラ[illegible]を作製した。ライブラリーをCOS細胞で一過性発現させ、JTT1抗体を用[illegible]ニングによりスクリーニングを行った。パニングにより濃縮されたcDNAの塩基[illegible]ジデオキシン法により決定した。フローサイトメトリー解析：細胞を抗体で[illegible]、FITC-抗マウスIgで染色後、フローサイトメーターで解析を行った。

【結果及び考察】FTL435細胞[illegible]マウスに免疫し、FTL435細胞のJTT.1抗体による凝集阻害を指標にモノクロー[illegible]抗体を作製し、JTT.2を得た。JTT.2抗体のアイソタイプはIgG1κであった。JTT[illegible]原は、FTL435 細胞、胸腺細胞や活性化リンパ球に強い発現が見られた。免疫[illegible]の結果、JTT.2抗原の分子量は還元下で24kDa と 28kDa の分子であった。この[illegible]JTT.1抗原の[illegible]。このことからJTT.2抗体は JTT.1 抗原を[illegible]胸腺細胞の[illegible]プレート上に固定したJTT[illegible]胸腺細胞は無刺激の状態では[illegible]プレート上のJTT.1抗原[illegible]によって阻害された。以上の[illegible]

[illegible]一は、JTT.1抗原を発現しているラ[illegible]cDNAライブラリーをCOS細胞で一過性発現させ[illegible]とにより 0.9kbp の遺伝子がクロ[illegible]COS 細胞は、JTT.1及びJTT.2 抗[illegible]から予想されるアミノ酸配列中には、精製JTT.1抗原のN[illegible]と一致する配列が存在していた。

以上の結果より、我々がクローニングした遺伝子は、JTT.1 抗原遺伝子であると考えられる。予想されるアミノ酸配列中には細胞外ドメインに2箇所の糖鎖結合部位、細胞内ドメインにPKCリン酸化[illegible]K 2リン酸化部位が2箇所存在していた。なおアミノ酸配列全長[illegible]一検索を行ったが、既知の分子の中には相同性のある分子は確認[illegible]から JTT.1 抗原は、新規の接着分子であると考えられる。

まとめ

1. JTT.1抗体により誘導される未知の[illegible]を阻害するJTT.2抗体を作製した。

2. JTT.2抗体は、JTT.1抗原を認識し[illegible]と考えられた。

3. JTT.1抗体刺激したラット胸腺細胞[illegible]1抗原に結合した。
この結合は、JTT.2抗体によって特[illegible]阻害された。

4. JTT.1抗原遺伝子を発現クローニング[illegible]取得した。
ホモロジー検索の結果、相同性の[illegible]は確認されなかった。

以上の結果からJTT.1抗原は、新規の[illegible]子であると考えられる。

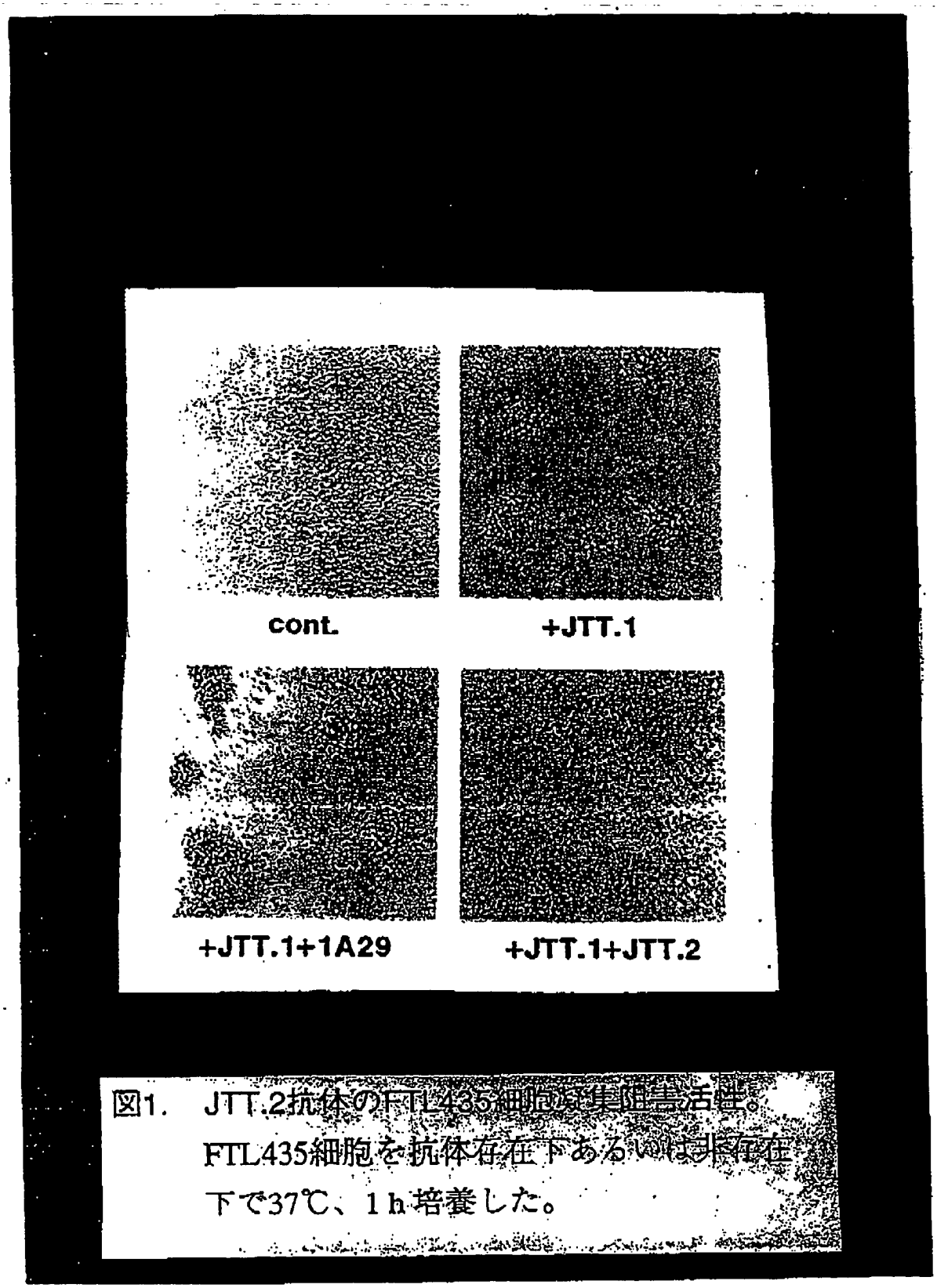


図1. JTT.2抗体のFTL435細胞凝集阻害活性。
FTL435細胞を抗体存在下あるいは非存在下で37℃、1h培養した。

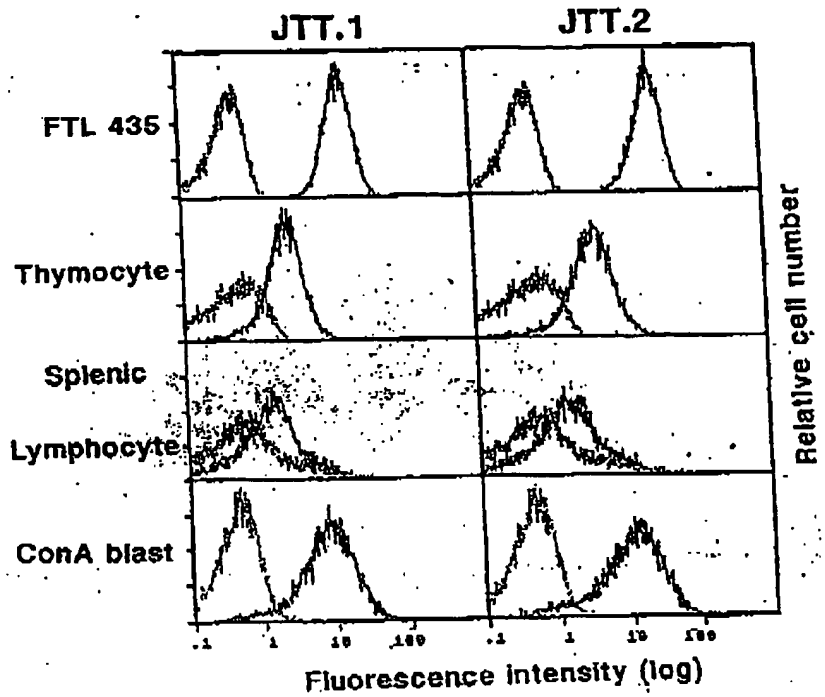


図2．各種細胞におけるJTT.1抗原とJTT.2抗原の発現パターンの比較。
FTL435、胸腺細胞、脾臓リンパ球、ConA blast を抗体で染色し、フローサイトメーターで解析した。

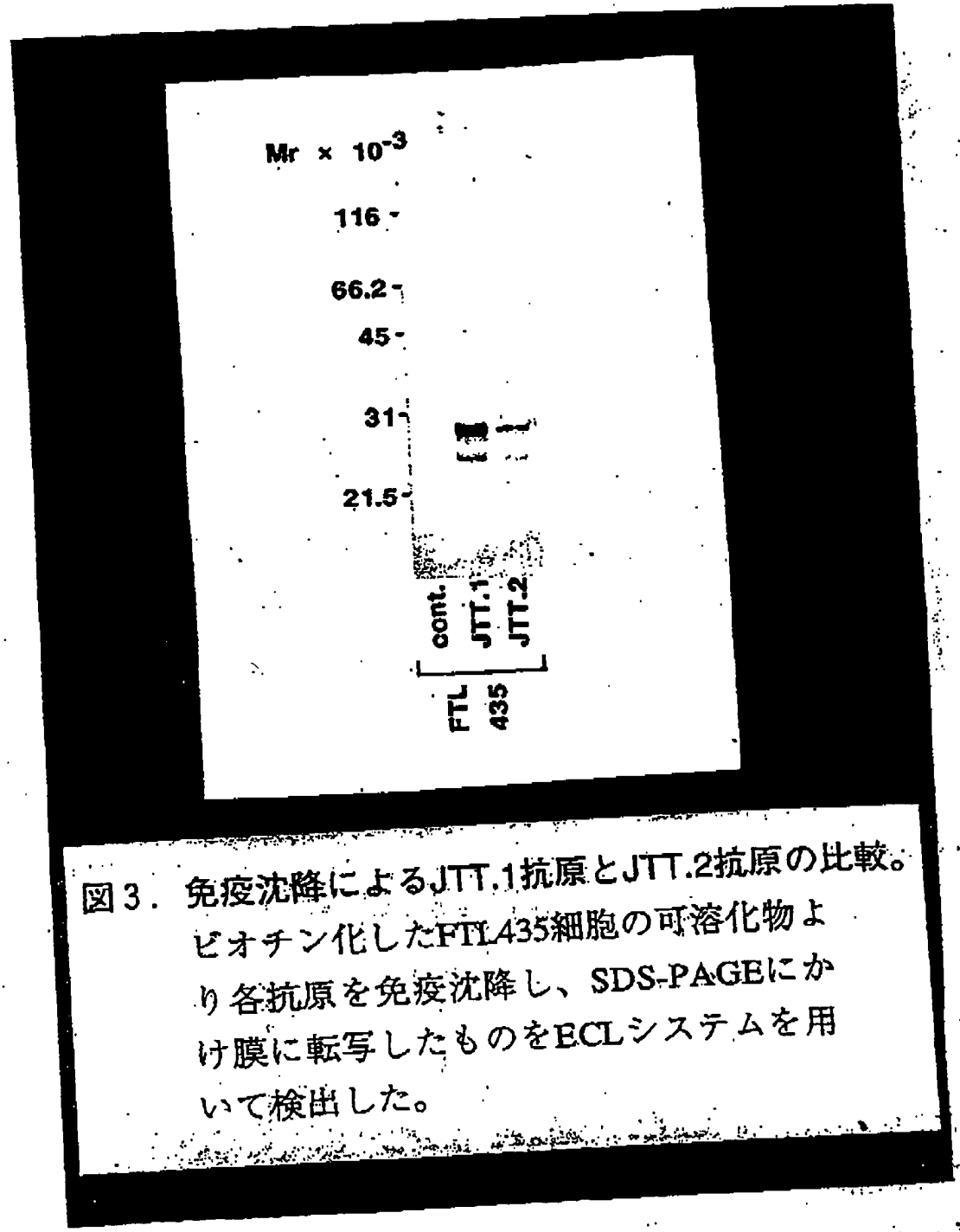


図3．免疫沈降によるJTT.1抗原とJTT.2抗原の比較。ビオチン化したFTL435細胞の可溶化物より各抗原を免疫沈降し、SDS-PAGEにかけ膜に転写したものをECLシステムを用いて検出した。

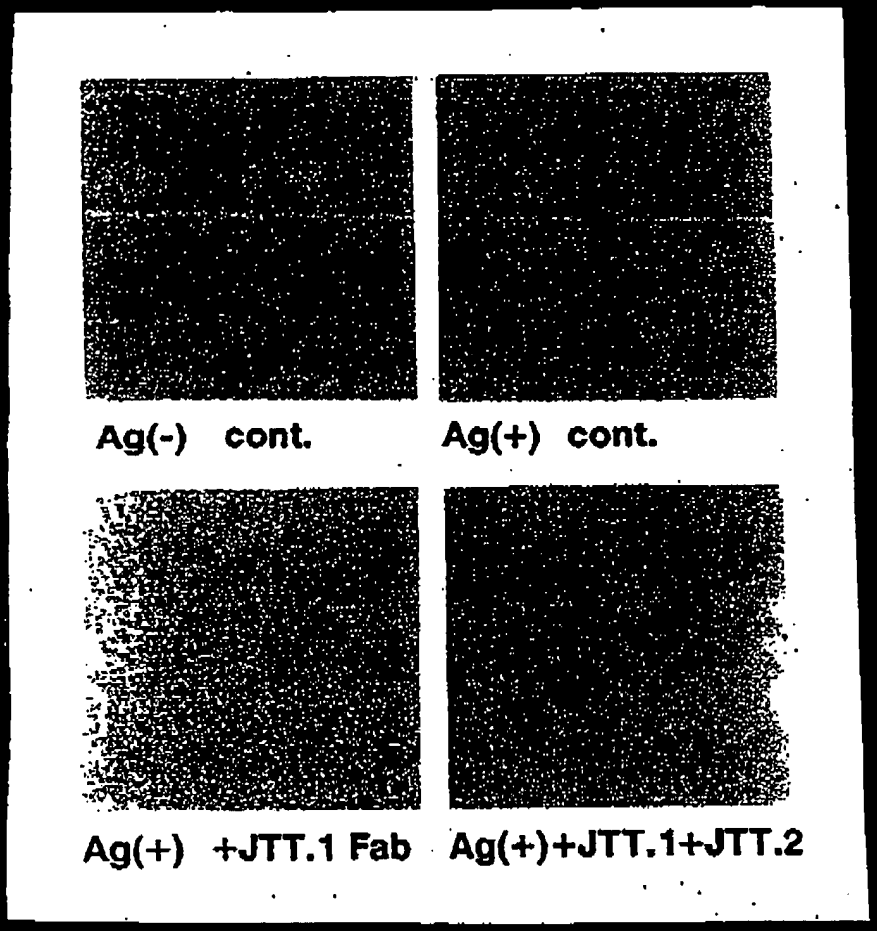


図４．JTT.2抗体は胸腺細胞のJTT.1抗原への結合を阻害する。
精製JTT.1抗原をプレートにコートし、抗体存在下あるいは非存在下でラット胸腺細胞を37℃、１ｈ培養した。

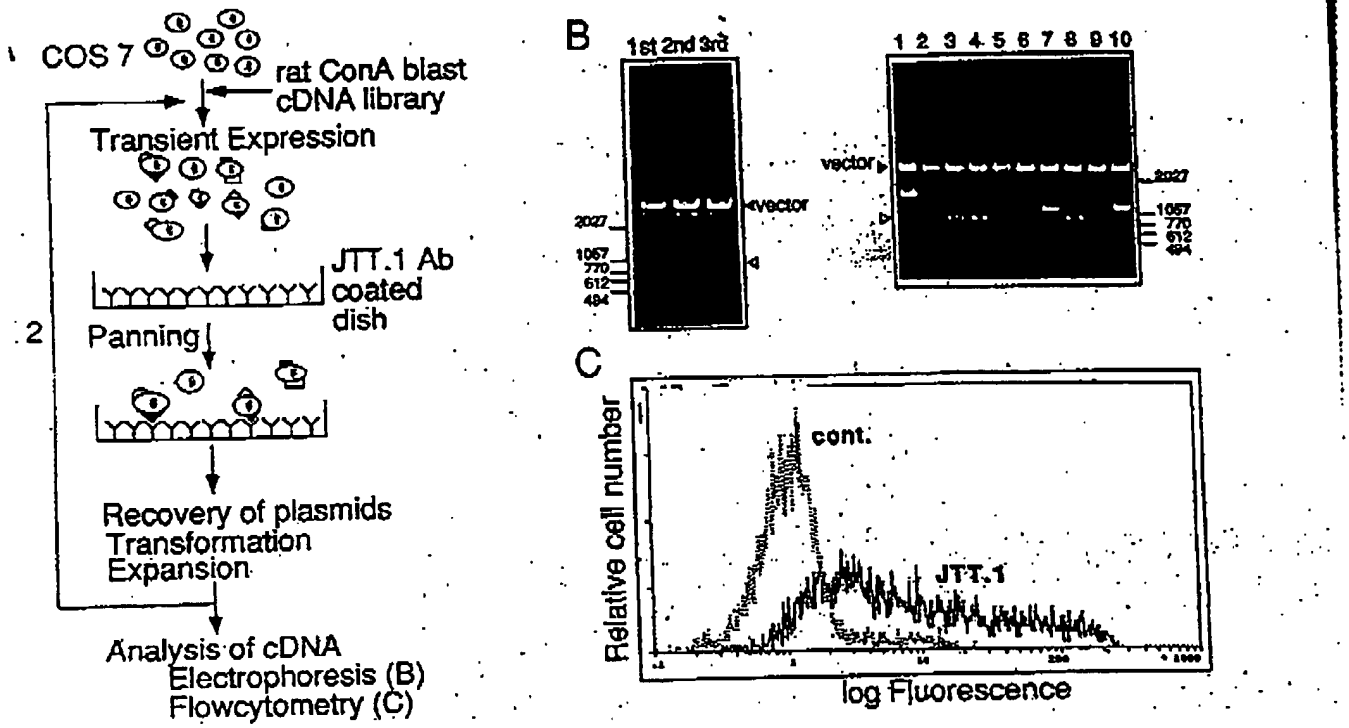
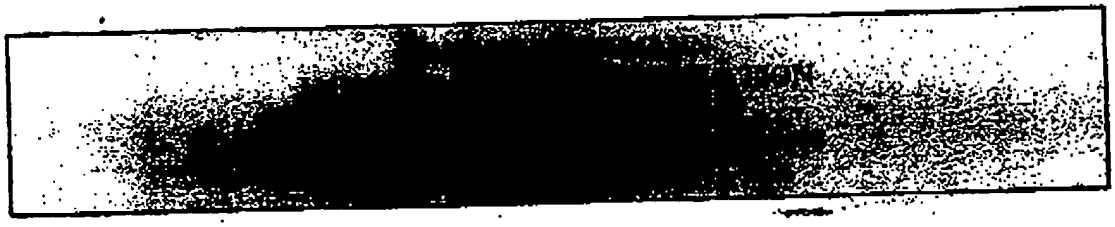


図５．JTT.1抗原の発現クローニング。

（A）パニング法によるcDNAクローニングの概略

（B）COS細胞より回収されたプラスミドの解析：回収されたプラスミドを制限酵素消化後、１％アガロースゲル電気泳動を行い、インサートDNAを解析した。

（C）JTT.1抗原cDNA産物とJTT.1抗体との反応性：パニング法により得られたJTT.1抗原cDNAを一過性発現させたCOS細胞をJTT.1抗体で染色し、フローサイトメーターで解析した。



TRANSLATION from Japanese to English

CERTIFICATE OF ACCURACY

This day personally appeared before me Gregor Hartmann, who after being duly sworn deposes and states:

that he is a translator of the Japanese and English languages, a professional provider of translations, accredited by the American Translators Association for Japanese to English translation;

that he is thoroughly familiar with these languages and has carefully made and verified the attached translation from the original document in the Japanese language, to wit:

Untitled article on research on monoclonal antibodies (JTT.1, JTT.2)

and that the attached translation is a true and correct English version of the original to the best of his knowledge and belief.

Gregor Hartmann

Gregor Hartmann, Translator

Sworn to before me

this date: 12/22/02

Ivana Bressler

Notary Public

Ivana Bressler
Notary Public of New Jersey
My Commission Expires 5/30/2007